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Bone Cellular Activity Associated with Skeletal Atrophy from Disuse.* (28672)

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Disuse of the skeletal system results in its atrophy with development of osteoporosis. Mechanical stresses and strains seem essential for normal bone formation. If these are absent, osteoblastic activity is reduced while resorptive processes continue at a normal pace resulting in a net loss of bone. However, little is known concerning the specific metabolic behavior of bone cells during osteoporosis of disuse.

Investigations with immobilized subjects generally show that the degree of atrophy is proportional to the extent of non-use. Such studies(1,2,3) have been based on urinary excretion of calcium and phosphorus, loss of bone ash weight or a decrease in bone breaking strength after selected periods of immobilization. In the current study the metabolic activity of bone cells during bone loss resulting from disuse was determined.

Methods. Young male rats of the Sprague-Dawley strain weighing 300-400 g were anesthetized with nembutal and the right rear leg immobilized with a plaster of paris cast. After one or 2 weeks of immobilization the rats were sacrificed and the tibia from each leg was removed, cleaned and weighed. The metaphyseal area of this bone and that from the distal end of the femur were split and washed in iced Krebs-Ringer bicarbonate medium, blotted dry with filter paper and weighed on a Roller-Smith balance. The samples were then placed in incubating flasks containing iced medium and stored on ice until the start of incubation. Similar samples were saved for cell nitrogen and citric acid determinations.

All samples were incubated in Warburg flasks at a temperature of 37°C for 3 hours. Approximately 100 mg of wet bone were used in each flask. Calcium-free Krebs-Ringer bicarbonate media buffered to a pH 7.4 was used. The 3 ml sample also contained 2 mg of glucose per ml as the substrate and 5 U of penicillin and 0.01 mg per ml of streptomycin were added to prevent bacterial growth. The solution was gassed with a 5% CO_2-95% O_2 mixture for 10 minutes before the bone sample was added and just prior to incubation the flask was gassed with the same mixture.

Following incubation, aliquots of the media were analyzed for citric acid by a modification of Natelson *et al.*(4), glucose by the method of Huggett and Nixon(5) and lactate by the Barker and Summerson(6) technique. Bone cellular content was estimated by determining the non-collagenous nitrogen level employing a modification of Lilienthal *et al.* method(7) as described by Borle *et al.* (8).

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TABLE I. Skeletal Response to Immobilization.

Treatment		Cell nitrogen, mg/100 mg wet bone	Tibia wet wt, mg	Bone citric acid, μg/100 mg wet bone
Control 1 wk	(11)*	.97 ± .08†	546 ± 24	275 ± 15
Immobilized 1 wk	(11)	.64 ± .05‡	536 ± 25	348 ± 13‡
Control 2 wk	(9)	.84 ± .05	634 ± 9	269 ± 25
Immobilized 2 wk	(9)	.68 ± .07‡	587 ± 11‡	251 ± 19

* No. of animals observed.
† Mean ± standard error of mean.
‡ Different at 99% confidence level.

Results. The immobilized limbs showed a significant drop in cell nitrogen after one week and after 2 weeks (Table I). Of the 20 rats observed, all showed a higher value of cell N_2 for the control leg than for the restricted. The control value at one week agreed with that previously reported(8) but decreased somewhat in this group during the 2 week study. It is possible that this difference was due to age and weight since the latter group was approximately 100 g heavier at time of experiment. Tibia weight did not decrease until the second week of immobilization, suggesting that the loss in bone material which occurs during non-use is preceded by a bone cellular change.

Bone citric acid values for the immobilized limbs were significantly greater at one week (Table I). At 2 weeks this difference had disappeared. The lactic acid output by incubating tissue (Table II) was also different at one week but not at 2 weeks. Whether a relationship existed between these 2 variables cannot be ascertained. The citric acid level of bone tissue consists of that adsorbed on the crystal lattice, of unknown origin, as well as that within the cell mitochondria. On the other hand, the lactic acid source would probably be restricted to that from the glycolytic cycle of the cell cytoplasm during incubation.

Glucose utilization was greatly increased in bones taken from the immobilized limbs (Table II) at both 1 and 2 weeks. Furthermore, it appeared that an inverse relationship existed between amount of cell nitrogen extracted and uptake of glucose. A calculation of this relationship (Table II) showed that these factors were correlated in the tissue from immobilized limbs at both time intervals and at 2 weeks in the controls. The citric acid content of the incubation media was also determined. Only trace amounts were found, which substantiates the findings of Borle *et al.*(8,9) that the output of lactic acid was some 30 times greater than that of citric acid under similar conditions.

Discussion. Under steady physiological conditions the predominant bone cell present will depend on whether bone resorption or formation is taking place. In these experiments it was evident that bone resorption had been initiated; thus one would expect to find evidence of osteoclastic activity. Several observations suggest that this assumption is correct. For example the increased bone citric acid content noted at one week is consistent with the evidence that parathyroid activity(10) is associated with bone resorption and that an increase in bone citric acid (11) will accompany such a response. Further, the rise in lactic acid output by incubating bone (Table II) was similar to the results of Borle *et al.*(9) who observed a

TABLE II. Effect of Immobilization on Bone Metabolism.

Treatment	Glucose utilization, μg/hr/mg cell N	Media lactic acid, μg/mg cell N	Cell N/glucose utilization, "r" value
Control 1 wk*	467 ± 28†	54 ± 3	−.203
Immobilized 1 wk	872 ± 86‡	106 ± 5‡	−.662§
Control 2 wk	619 ± 78	72 ± 6	−.651§
Immobilized 2 wk	906 ± 89§	71 ± 4	−.826‡

* † ‡ See Table I. § Different at 95% confidence level.

marked change in this factor from animals injected with parathyroid extract. The fact that both of these responses had disappeared at 2 weeks when a definite reduction in bone weight had occurred may indicate the active phase of bone resorption was completed and the bone had adapted to a different physiological state.

The marked decrease in cell nitrogen which preceded bone atrophy indicated that disuse resulted in a decrease of cell number or a change in cell type. The explanation for the reduced cellular nitrogen cannot be readily given as a shift from a predominately osteoblastic to osteoclastic population in view of the results of Borle *et al.* which showed that estrogenic treatment resulted in a reduced cell nitrogen content. It has been shown that estrogenic treatment(12) results in extensive new bone formation in the metaphyses of the long bones indicating a predominately osteoblastic population.

The greatly increased glucose utilization value for the immobilized bone may be interpreted in 2 ways. It may be that the reduction in cell nitrogen was indicative of a reduced number of cells which became highly active or it could mean a change to another cell type of a higher metabolic activity. It is also possible that the energy forming substances of the bone tissue were decreased during immobilization and the increased utilization of the substrate glucose was merely a matter of deficiency.

It seems probable that bone atrophy resulting from disuse is an active process which is dependent on a change in either cell number or type, a change which precedes the actual loss in skeletal material. It remains to be determined whether this function is mediated *via* the parathyroid gland, although some of the results of the present study indicate that such is the case.

Summary. Bone samples from control and immobilized rat legs were subjected to metabolic and chemical studies. Immobilization of the limb resulted in a marked drop in cellular nitrogen within one week, an effect which was still evident at 2 weeks. Loss in bone weight did not occur until the second week indicating that the reduction in cell nitrogen preceded bone resorption. The possibility that bone atrophy resulting from disuse was mediated *via* the parathyroid gland was advocated in view of the increased level of bone citric acid content and the higher output of lactic acid by the incubating bone tissue from the immobilized limbs.

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